Isolation of Two Quinochalcones from *Carthamus tinctorius*

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Red and yellow colorants from the safflower florets (*Carthamus tinctorius* L., Compositae) are applicable to processed foods and soft drinks as color additives, cosmetic rouge, dyes, and medicine [Meselhy *et al.*, 1993]. Almost all of these red and yellow colorants in the petals of safflower are classified as members of the *C*-glucosylquinochalcone family of flavonoids that occurs only in *C. tinctorius* [Kazuma *et al.*, 2000]. A red colorant of the safflower, carthamin, is composed of two *C*-glucosylquinochalcone moieties [Obara and Onodera, 1979]. Two major yellow *C*-glucosylquinochalcone colorants of safflower are hydroxysafflor yellow A and safflor yellow B [Takahashi *et al.*, 1984; Meselhy *et al.*, 1993]. Besides these two, several other minor quinochalcone colorants, including safflomin A [Onodera *et al.*, 1981], safflor yellow A [Takahashi *et al.*, 1982], safflomin C [Onodera *et al.*, 1989], tinctormin [Meselhy *et al.*, 1993], precarthamin [Kumazawa *et al.*, 1994], anhydrosafflor yellow B [Kazuma *et al.*, 2000], and cartormin [Yin and He, 2000] have been reported. As a part of our ongoing search for chemical constituents from *C. tinctorius*, we isolated five red and yellow quinochalcones (carthamin, hydroxysafflor yellow A, safflor yellow B, precarthamin, and cartormin) and examined their pH and thermal stability, and antioxidant activity [Kim and Paik, 1997; Yoon *et al.*, 2003; Yoon *et al.*, 2007]. Enzymatic conversion of the yellow colorant precarthamin into the red pigment carthamin was also performed [Cho *et al.*, 2000]. Our continuing work led to the isolation of safflomin C and a related *C*-glucosylquinochalcone from the BuOH-soluble fraction of the safflower florets. This paper describes the procedures of isolation and the structural elucidation of these two quinochalcones **1** and **2** (safflomin C and isosafflomin C, Fig. 1).

The dried petals of *C. tinctorius* (3 kg) were extracted at room temperature with 80% aqueous MeOH (10 L×2). After filtration and evaporation of the solvent under reduced pressure, the combined crude extract was suspended in water and then successfully partitioned with EtOAc and BuOH to afford EtOAc-soluble and BuOH-soluble fractions. The BuOH-soluble fraction was subjected to Sephadex LH-20 (5.5×15 cm) using 20~70% CH_3CN/H_2O gradient to yield five fractions. The fourth fraction (1.0 g) was subjected to reversed phase HPLC (20~40% CH_3CN/0.1% TFA-H_2O, ODS column; 250×10 mm, flow rate; 2 mL/min) to afford two peaks of typical UV absorption at about 400 nm, suggesting the presence of quinochalcones. The repeated collection of each peak (28.107 and 30.093 min) gave *C*-glucosylquinochalcones **1** (86.3 mg) and **2** (50.9 mg).

Compound **1**: mp 171.3°C; $[\alpha]_D$ -99.1 (*c* 0.1, MeOH); UV (MeOH) 406 (log ε 4.37), 346 (sh), 230 (log ε 4.26) nm; IR (KBr) 3365, 1669, 1600 cm^{-1}; FABMS *m/z* 615.2

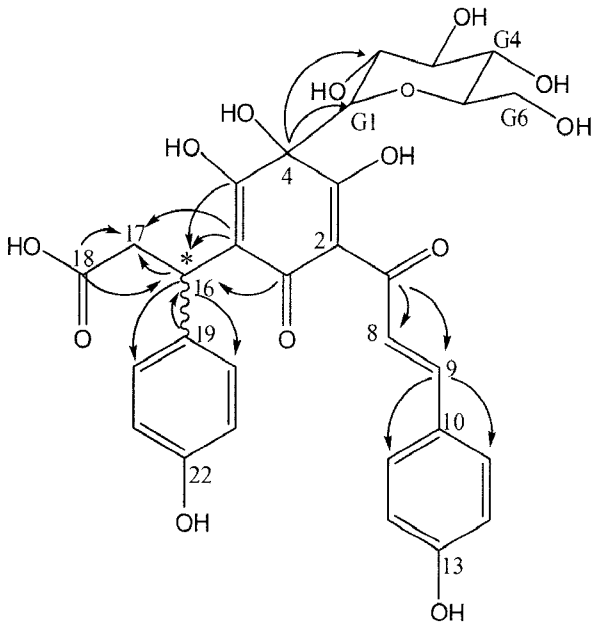

Fig. 1. Structures of 1 (safflomin C) and 2 (isosafflomin C) with opposite configuration relationship at C-16 and selected HMBC (C→H) correlation.

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Abbreviations: FABMS, fast atom bombardment; HMBC, heteronuclear multiple bond correlation; HPLC, high performance liquid chromatography

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[M+H]$^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (1H, d, *J*= 16.0 Hz, H-9), 7.50 (2H, d, *J*=8.4 Hz, H-11, 15), 7.43 (1H, d, *J*=16.0 Hz, H-8), 7.20 (2H, d, *J*=8.4 Hz, H-20, 24), 6.79 (2H, d, *J*=8.4 Hz, H-12, 14), 6.65 (2H, d, *J*=8.4 Hz, H-21, 23), 4.72 (1H, dd, *J*=9.2, 7.2 Hz, H-16), 3.69 (1H, dd, *J*=11.6, 2.0 Hz, H-G6), 3.56 (1H, br d, *J*=11.6 Hz, H-G6), 3.48 (1H, overlapped, H-G1), 3.48 (1H, overlapped, H-G2), 3.37 (1H, t, *J*=9.6 Hz, H-G4), 3.22 (1H, dd, *J*=15.2, 9.2 Hz, H-17), 3.22 (1H, m, H-G3), 3.03 (1H, dd, *J*=15.2, 7.2 Hz, H-17), 2.97 (1H, m, H-G5); ^{13}C NMR (100 MHz, CD_3OD) see Table 1.

Compound **2**: mp 175.0°C; $[\alpha]_D$ -114.7 (*c* 0.1, MeOH); UV (MeOH) 407 (log ε 4.53), 348 (sh), 230 (log ε 4.44) nm; IR (KBr) 3354, 1670, 1600 cm^{-1}; FABMS *m/z* 615.2 [M+H]$^+$; ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (1H, d, *J*= 16.0 Hz, H-9), 7.49 (2H, d, *J*=8.4 Hz, H-11, 15), 7.43 (1H, d, *J*=16.0 Hz, H-8), 7.18 (2H, d, *J*=8.4 Hz, H-20, 24), 6.78 (2H, d, *J*=8.4 Hz, H-12, 14), 6.63 (2H, d, *J*=8.4 Hz, H-21, 23), 4.77 (1H, dd, *J*=9.2, 6.4 Hz, H-16), 3.75 (2H, overlapped, H-G6), 3.53 (1H, overlapped, H-G1), 3.53 (1H, overlapped, H-G2), 3.43 (1H, t, *J*=9.6 Hz, H-G4), 3.30 (1H, overlapped, H-17), 3.26 (1H, overlapped, H-G3), 3.06 (1H, m, H-G5), 2.98 (1H, dd, *J*=16.0, 6.4 Hz, H-17); ^{13}C NMR (100 MHz, CD_3OD) see Table 1.

The FABMS of compound **1** showed the peaks at *m/z* 615.2 [M+H]$^+$. The UV absorption maxima at 406, 346 (sh), and 230 nm, and together with the IR absorption bands at 3365, 1669, and 1600 cm^{-1} suggested that **1** was a quinochalcone glycoside. In the ^{1}H NMR spectrum of **1**, two trans-olefinic protons at δ 7.70 (1H, d, *J*=16.0 Hz, H-9) and δ 7.43 (1H, d, *J*=16.0 Hz, H-8) and aromatic protons of two AA'BB' spin systems at δ 7.50 (2H, d, *J*= 8.4 Hz, H-11, 15) and 6.79 (2H, d, *J*=8.4 Hz, H-12, 14) and at δ 7.20 (2H, d, *J*=8.4 Hz, H-20, 24) and 6.65 (2H, d, *J*=8.4 Hz, H-21, 23) were observed. One *C*-glucosyl anomeric proton resonated at δ 3.48 (1H, overlapped with H-G2) was connected with six sugar protons of the glucosyl moiety at δ 3.48 (H-G2), 3.22 (H-G3), 3.37 (H-G4), 2.97 (H-G5), and 3.69 and 3.56 (2H, H-G6). In the HMBC spectrum of **1**, the presence of the following cross peaks were observed: C-1 (δ 192.28), C-5 (δ 173.20), C-6 (δ 113.84), C-17 (δ 38.72), C-18 (δ 176.62), C-19 (δ 135.52), C-20, 24 (δ 129.45) with H-16 (δ 4.72); C-6 (δ 113.84), C-16 (δ 36.65), C-18 (δ 176.62), C-19 (δ 135.92) with H-17 (δ 3.03 and 3.22). These ^{1}H and ^{13}C NMRs and HMBC data of **1**, with the characteristic absorption maximum at 406 nm, were consistent with the structure of safflomin C [Onodera *et al.*, 1989].

Spectroscopic data of compound **2** (R_t 30.093 min) were very similar to those of compound **1** (R_t 28.107 min). The FABMS of **2** showed the peaks at *m/z* 615.2 [M+H]$^+$. The UV absorption maxima were detected at 407, 348 (sh), and 230 nm, and the IR absorption bands were at 3354, 1670, and 1600 cm^{-1}. In the ^{1}H NMR spectrum of **2**, two trans-olefinic protons at δ 7.70 (1H, d, *J*=16.0 Hz, H-9) and δ 7.43 (1H, d, *J*=16.0 Hz, H-8) and the aromatic protons of two AA'BB' spin systems at δ 7.49 (2H, d, *J*=8.4 Hz, H-11, 15) and 6.78 (2H, d, *J*=8.4 Hz, H-12, 14) and at δ 7.18 (2H, d, *J*=8.4 Hz, H-20, 24) and 6.63 (2H, d, *J*=8.4 Hz, H-21, 23) were observed. One *C*-glucosyl anomeric proton resonated at δ 3.53 (1H, overlapped with H-G2) was connected with six sugar protons of the glucosyl moiety at δ 3.53 (H-G2), 3.26 (H-G3), 3.43 (H-G4), 3.06 (H-G5), and 3.75 (2H, H-G6). The ^{13}C NMR data of **1** and **2** were essentially the same except for the values at C-17 (**1**; δ 38.72, **2**; δ 37.56) and C-16 (**1**; δ 36.65, **2**; δ 36.19) (Table 1). In addition, the following cross peaks were observed:from the HMBC spectrum of **2** (Table 1): C-1 (δ 192.11), C-5 (δ 172.89), C-6 (δ 113.86), C-17 (δ 37.56), C-18 (δ 176.48), C-19 (δ 135.53), C-20, 24 (δ 129.51) with H-16 (δ 4.77); C-6 (δ 113.86), C-16 (δ 36.19), C-18 (δ 176.48), and C-19 (δ

Table 1. ^{13}C NMR and HMBC Spectral Data of Compounds 1 and 2 in CD_3OD

carbon	1		2	
	^{13}C	HMBC (C→H)	^{13}C	HMBC (C→H)
1	192.28	16	192.11	16
2	108.39		108.60	
3	195.54		195.59	
4	82.12	G1, G2	82.12	
5	173.20	16	172.89	16
6	113.84	16, 17	113.86	16, 17
7	180.36	8, 9	180.39	8, 9
8	119.25	9	119.25	
9	143.34	11, 15	143.31	11, 15
10	128.22	8, 9, 12, 14	128.22	8, 9, 12, 14
11, 15	131.49	9, 12, 14	131.49	9
12, 14	116.68	11, 15	116.67	11, 15
13	161.07	11, 12, 14, 15	161.09	11, 12, 14, 15
16	36.65	17, 20, 24	36.19	17, 20, 24
17	38.72	16	37.56	16
18	176.62	16, 17	176.48	16, 17
19	135.52	16, 17, 21, 23	135.53	16, 17, 21, 23
20, 24	129.45	16, 21, 23	129.51	16
21, 23	115.49	20, 24	115.58	20, 24
22	156.29	20, 21, 23, 24	156.32	20, 21, 23, 24
G1	88.23	G2	88.23	G2
G2	70.44	G1, G3	70.45	G1, G3
G3	79.57	G2, G4	79.59	G2, G4
G4	69.75	G3, G6	69.68	G3, G6
G5	80.63	G4, G6	80.57	G4
G6	61.32	G4	61.30	G4

135.53) with H-17 (δ 2.98 and 3.30). These resemblance and difference observed in ^{1}H and ^{13}C NMR and HMBC data between **1** and **2** allowed the identification of the structure of **2** as isosafflomin C with opposite configuration relationship at C-16. Although the presence of **2** (isosafflomin C) has been reported earlier as an abstract (Poster section, B5 12) by *Sato et al.* [1998], no detailed spectroscopic data including ^{1}H and ^{13}C NMR data have been published. They described that the absolute configurations of safflomin C and isosafflomin C derivatives (hydrolysis followed by methylation products) at C-16 are R or S. In this paper, we describe the isolation and chemical structure determination of safflomin C and isosafflomin C from *C. tinctorius* with detailed ^{1}H and ^{13}C NMR and other spectroscopic data.

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